ISOLATION OF ATTINI BROOD FROM THE SOCIAL ENVIRONMENT (HYMENOPTERA: FORMICIDAE)[1]

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ABSTRACT: Brood from several species of the fungus-growing Attini ants, including *Atta colombica tonsipes, Atta cephalotes* and *Atta sexdens,* were isolated from the social environment and artifically reared on sterile agar plates. Though isolate larvae would accept fungal food on the end of a needle and subsequently pupate, isolate brood seemed unable to fully develop to adulthood even with laboratory imitation of adult worker care. This was due to the inability of pupae to emerge from the pupal skin, and to the consistent onset of contamination. By contrast, brood in control plates consisting of workers and bits of fungus garden as well as brood, developed to adulthood. This apparently occurred since contamination was inhibited by the social environment and because worker licking and manipulation removed pupal coverings. An anomalous condition, however, arose in one *Atta cephalotes* control where emerging callows retained abnormally pale coloration. Developmental coloration of isolate pupae was also studied, with eyes noted as darkening first, followed by the mandibular masticatory border, and, finally, the head and gaster, the results conforming to previous studies.

DESCRIPTORS: Attini, brood, isolation, social environment, contamination, pupa, coloration.

It has frequently been noted that ant broods cannot develop to adulthood in absence of the social environment (Wilson, 1972). This is particularly true of the fungus-growing Attini ants (Weber, 1966 a, b). The present studies were undertaken primarily to determine the possibility of rearing Attini brood to adulthood in isolation from the social environment. The development of such a technique would aid in the determination of the factors of social care necessary for complete brood development, and would have implications for genetic and caste research. Also desired were close observations of the developmental color changes which occur during the pupal and callow stages.

Attini brood (worker larvae and pupae only) were taken from the laboratory colonies of Dr. Neal Weber, Swarthmore College, Swarthmore Pennsylvania, and isolated on petri dishes of sterile 1% non-nutrient agar. A grid was superimposed on the bottom of each dish, and one piece of brood placed on each grid box. This was designed to aid in the observation of each specific developing larva or pupa. A method was devised (after Weber, 1972) to feed isolate larvae by placing small fragments of fungal hyphae on the tip of a dissecting needle and thrusting the clump at the larval mouthparts. Several isolate larvae responded to such feeding, two

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of which subsequently pupated. Pupal pigmentation was noted until the onset of movement within the pupal covering. The removal of the exuviae was unseccessfully attempted by stroking the skin with a moist, fine paintbrush while tugging with a dissecting needle.

Two experimental series were run, the isolates being placed on petri dishes, while controls of bits of fungus garden, workers and brood taken from the same laboratory colonies as the isolates were also placed on petri dishes. This was designed to determine (1) whether any inherent contamination or infection would prevent brood development even in the presence of social care, and (2) to compare worker brood development with and without the benefits of the social environment. Series #1 consisted of two petri dishes of control material containing approximately five grams garden, five media workers, five minima workers and several pupae embedded in the garden fragments. Materials for both dishes were taken from two separate *Atta colombica tonsipes* laboratory colonies. Isolate brood from the same colonies were placed in numbered grid boxes of two petri dishes. Dish #1 contained five isolate larvae, and dish #2 five isolate pupae. Series #2 utilized material from three Attini species, *Atta colombica tonsipes, Atta cephalotes,* and *Atta sexdens.* Each control dish and isolate brood dish was taken from the same laboratory colony. Thus, three controls were established, controls A, B, C respectively. Control A contained five to seven grams garden, five media and two larvae of *Atta colombica tonsipes.* Control B consisted of five grams garden, five media, five minima, two pupae and one larva of *Atta cephalotes,* while control C contained five to six grams garden, six minima, two media and two larvae of *Atta sexdens.* Isolate dish #1 held four *tonsipes* larvae only one of which responded to artificial feeding efforts. Isolate dish #2 consisted of six *cephalotes* pupae in varying degrees of development. Isolate dish #3 contained two *sexdens* pupae and two larvae.

RESULTS

Results of series #1 appear in tables 1 and 2, where feeding, coloration development and contamination are tabulated against progressive dates of observation of isolated brood. In dish #1, two larvae pupated in isolation, one of them after two periods of artificial feeding. Streaks of brown were noted in the three larvae that responded to feeding and are possibly the gut full of food or uric acid metabolites. Though larvae would pupate in isolation

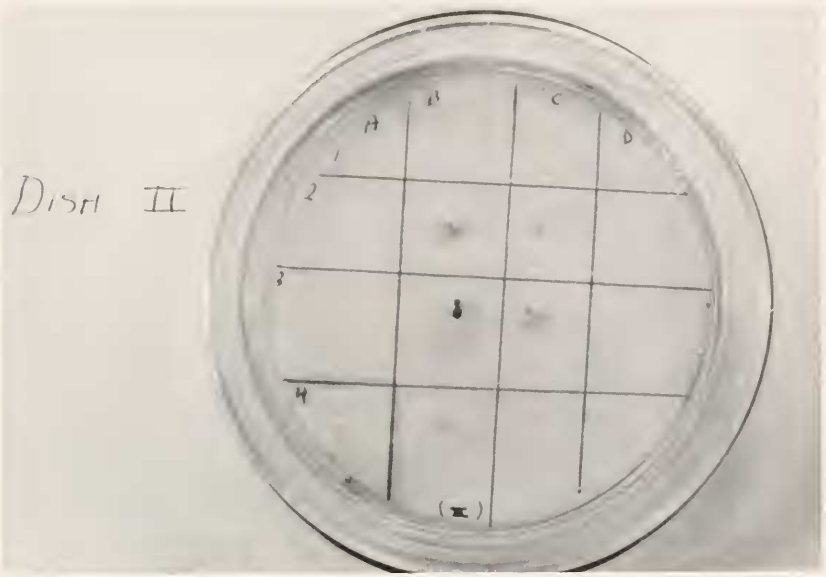

Figure 1a. Dish #2 series #1. The pupa in the center (grid box #3B) was moving at time of photographing–see Fig.2.

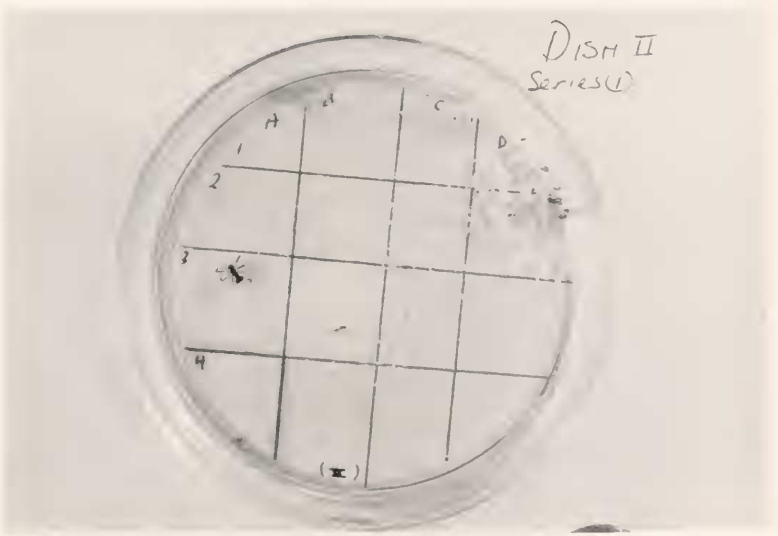

Figure 1b. Dish #2 series #1 after adult *tonsipes* worker was introduced by the experimentor in an effort to induce nursing behavior after artificial attempts at removing the pupal skin had failed. Note how the worker has discarded the pupae and has excavated around a small piece of fungal contamination. The ant was dying at time of photographing. See text.

Figure 2. Pupa from dish #2 series #1. Note the dark eyes and dark masticatory border of the mandibles. The pupa was moving at time of photographing and would have emerged in the presence of licking by workers.

Figure 3. Semi-pupa from dish #1 series #1. Originally isolated as a larva, this *Atta colombica tonsipes* brood pupated several days after being fed fungus from the tip of a dissecting needle.

Figure 4. Close-up of control B, series #2, *Atta cephalotes*. Note the pale head of the media (in the upper center) which hatched in the control, an anomaly which remained until the media's death. A normal worker appears in the lower center.

Figure 5a. A typical control dish with workers and garden fragment after one week of isolation from the main colony. Extensive excavation of agar and exhausted substrate particles are evident.

from worker care, contamination occurred in all cases before the brownish coloration of a developing pupa began. Data of pupal development in dish #2 are found in table 2. One of the pupae became contaminated soon after isolation. Pupa #3B was constantly moving the tips of its limbs and antennae by March 1, as well as its mandibles. Eyes were blackish, the cutting edge of the mandibles dark brown. Unsuccessful attempts were made to release this pupa from its covering with a fine paintbrush and dissecting needle. Pupa #3C when poked moved slightly and exuded a small brownish droplet from the tip of its gaster. Since attempts to remove the exuviae failed, an adult *tonsipes* worker from the control dish was introduced to isolate dish #2 in an effort to induce nursing behavior. However, instead of removing

Figure 5b. A control dish one week after the workers had died. Contamination is extensive. Note the pupa embedded in the dying garden. This was the only case in which brood did not reach adulthood in the control dishes.

the pupal skins from the struggling pupae, the worker discarded the pupae within one day and proceeded to excavate agar around some fungal contamination in a manner similar to normal worker treatment of garden fragments in the control situation (see Fig. 1 a and b).

The second series was designed to increase gross numbers of brood utilized and to use brood from three different species to compare results across species. Results are tabulated in tables 3 and 4. Dish #1 of the second series (data not listed) contained four larvae none of which would respond to feeding. Larva #3B pupated after four days isolation. After six days complete contamination of the isolated brood occurred. All became soft and mushy with a yellowish-gray tinge. Dish #2 contained six pupae

(see table 3) four of which were free of contamination for nineteen days. Pupal coloration began first in the compound eyes which gradually deepened in their brown color. The mandibular masticatory border assumed pigmentation next, after the eyes had already taken on a dark brown hue. Coloration of the head and gaster followed until the entire pupa had a dark brown pigmentation and movement began (see Fig. 2). Several of the pupae were covered with strands of fungus, but this may have been species specific fungus and not contaminants (Weber, 1966b). By March 14 slight movement was observed in pupa #3B, but it became contaminated before any attempts at removing the pupal skin were performed. By March 20 all the isolate pupae in this dish had become contaminated. Dish #3 consisted of two larvae and two pupae. The larvae did not feed though pupating after two days isolation. The two pupae were immature and though eye coloration deepened, their body coloration remained white throughout

TABLE 1

FIVE ISOLATE LARVAE, *Atta colombica tonsipes*, FIRST SERIES, DISH #1

DATE	RESPONSE TO FEEDING					BODY COLORATION					CONTAMINATION				
	1C	2B	2C	3B	3C	1C	2B	2C	3B	3C	1C	2B	2C	3B	3C
2-13	+	–	+	–	+	W/B	W	W/B	W	W/B	–	–	–	–	–
2-18	–	–	–	–	+	W	W	W	W	W/B	–	–	–	+	–
2-22	–	–	–	–	+	W	W	W	W	W/B	–	–	–	+	–
2-27	–*	–**	–	–	–	W	W	W	W	W/B	–	–	+	+	+
3-1	–	–	–	–	–	W	W	W	W	W/B	–	–	+	+	+

W=white; B=brownish streak; + = positive response; – = negative response, *;** Pupation occurred.

TABLE 2

FIVE ISOLATE PUPAE, *Atta colombica tonsipes*, FIRST SERIES, DISH #2

DATE	EYE COLORATION					BODY COLORATION					CONTAMINATION				
	2B	2C	3B	3C	4B	2B	2C	3B	3C	4B	2B	2C	3B	3C	4B
2-13	B	W	Br	LBr	W	LBr	W	LBr	W	W	–	–	–	–	–
2-18		Br	B	DBr	Br		W	Br	W	W	+	–	–	–	–
2-22		DBr	B	DBr	Br		W	DBr	LBr	W	+	–	+	–	–
2-27*		DBr	B	DBr	DBr		LBr	DBr	LBr	W	+	–	+	–	–

*An adult *tonsipes* worker was introduced to the dish on March 1, See text.

W=white; B=black; Br=brown; LBr=light brown; DBr=dark brown; + = positive response; – = negative response.

TABLE 3

SIX ISOLATE PUPAE OF *Atta cephalotes;* SECOND SERIES, DISH #2*

DATE	EYE COLORATION						BODY COLORATION						CONTAMINATION					
	2B	2C	3B	3C	4B	4C	2B	2C	3B	3C	4B	4C	2B	2C	3B	3C	4B	4C
3-1	W	LBr	LBr	DBr	B	LBr	W	W	W	W	LBr	W	–	–	–	–	–	–
3-7	W	DBr	DBr	B	B	DBr	W	LBr	LBr	Br	Br	LBr	–	–	–	–	–	–
3-14	Br	B	B	B	B	B	LBr	Br	Br	DBr	DBr	B	–	–	+	+	–	–
3-20	Br	B	B	B	B	B	LBr	Br	Br	DBr	DBr	Br	+	+	+	+	+	+

W=white; Br=Brown; LBr=light brown; DBr=dark brown; B=black; + = positive; – = negative.

*Dish #1 became completely contaminated after six days; data not shown.

TABLE 4

TWO ISOLATE PUPAE‡ AND TWO ISOLATE LARVAE*OF *Atta sexdens;* SECOND SERIES, DISH #3

DATE	RESPONSE TO FEEDING		EYE COLORATION				BODY COLORATION				CONTAIMINATION			
	2C*	3B*	1B	2B	2C	3B	1B	2B	2C	3B	1B	2B	2C	3B
3-5	–	–	W	LBr			W	W	W	W	–	–	–	–
3-7	pupated		W	LBr	W	W	W	W	W	W	–	–	–	–
3-14			LBr	Br	W	W	W	W	W	W	–	–	+	–
3-20			Br	Br	W	W	LBr	W	W	W	+	+	+	–

*2C & 3B are larvae, ‡ 1B & 2B are pupae

On 3-20, 3Bwas returned to *Atta sexdens* Control C since artificial means of removing the pupal shell failed. See text.

W=white; Br=brown, LBr=light brown; + = positive; – = negative.

their isolation period. On March 20 one of the larvae which had pupated in isolation was returned to its control (C) in an effort to induce the adult *sexdens* control workers to resume nursing care. Though initially attracted to the pupa (including licking, nipping and other tactile manipulation), the ants soon discarded it.

CONTROL RESULTS

Series #1 controls survived in isolation from the larger colony approximately ten days after which the garden fragments became dessicated and workers died. No contamination of brood receiving control worker care occurred, and these workers were able to bring all but one piece of brood to adulthood before dying. No contamination was allowed to approach the garden since the control groups excavated agar in a circle around the garden fragment. Extensive contamination began to occur after one week and workers were unable to prevent the contamination from spreading. Pupal coloration under control conditions appeared no different than isolate developmental coloration mentioned previously. Series #2 controls utilizing three different Attini species, generally remained viable longer than series #1 controls. Control A (*Atta colombica tonsipes*) survived nineteen days, the ants excavating heavily around the garden fragment as well as around the edge of the petri dish. The garden fragment was maintained in a fluffy, healthy condition for over two weeks, while one pupa developed to the callow stage. All workers died by March 19. Control B *(Atta cephalotes)* had two pupae embedded in its garden fragment. One underwent color changes identical to those noted in dish #2 series #2. The workers excavated extensively around the edge of the petri dish as well as around the garden itself. Minima, unlike media, seldom ventured off the garden fragment onto the agar surface. One of the pupae ecdyced on March 11 and was licked extensively by both minima and media. The resultant callow (a media) was unable to walk for two days and was exceptionally pale. This callow subsequently darkened abnormally, its gaster and thorax assuming normal darkness, while its head remained pale (see Fig. 4). This anomalous condition contrasted sharply with the normal dark head coloration of the control medias. Another media callow emerged on March 20 again after extensive licking and manipulation by workers. By March 25, its head had also remained pale eventhough its gaster and other parts of its body had darkened normally. No contamination was

visible in this control until April 1. Control B remained viable a total of forty days and some of the workers were still alive at the time of this writing. Control C remained viable for over thirty-five days at which time the garden fragment began to deteriorate probably from lack of adequate substrate. However, several minima survived several days beyond garden deterioration. Workers (primarily media) excavated around the edges of the petri dish but not around the garden fragment.

DISCUSSION

Attempts at artificially rearing Attini brood were successful up to a point. Larvae would accept food and pupate in isolation from the social environment and pupae would develop to the pre-callow stage. Pupae, however, were unable to extricate themselves from their coverings without worker aid. All attempts to duplicate the licking and tactile manipulation of the workers failed. In addition, contamination invariably killed the isolate brood if inability to emerge from the pupal covering did not. The inability of the isolate brood to develop to adulthood seems to center around their susceptibility to contamination and their incapacity to emerge from the pupal skin without worker aid. By comparison, control brood development was uninhibited, and contamination in no cases affected or killed brood. Contamination in general took far longer to appear in the controls than in the isolates, and when it did was kept from proximity of the garden fragment in which the brood was embedded (Weber, 1956a, 1966a, 1972 and Martin, 1974). The brood apparently do not possess any inherent contamination-inhibiting qualities since contamination in the isolate dishes was unrestricted. Rather, the social environment seems instrumental in contamination inhibitation (Weber, 1966a). Coloration of pupae during development conformed to previous studies (Weber, 1966b), with the compound eyes darkening first, then the masticatory border of the mandibles, and finally the gaster and head. Upon emerging, callows were pale and weak and gradually darkened to normal coloration. In the *Atta cephalotes* control, callows retained a pale head long after the rest of the body had darkened to normal specie coloration. Since the original control material was taken from a laboratory colony collected

several years ago, it is possible that genetic mutation or type of substrate supplied may be affecting head coloration. Further study with this laboratory *Atta cephalotes* colony is indicated. Though the small numbers of brood and control material by no means show this to be conclusive, *Atta colombica tonsipes* isolate brood and controls remained viable for a significantly shorter time than either *Atta cephalotes* or *Atta sexdens* material.

SUMMARY

Ant brood particularly the Attini brood is totally dependent on social care for the complete development to the adult stage. Three species of Attini were used, *Atta colombica tonsipes, Atta cephalotes,* and *Atta sexdens.* Attempts at rearing Attini brood in isolation from the social environment failed not because of failure to induce larvae to eat, but because pupae were unable to emerge from the pupal skin and because contamination invariably occurred. Licking by the workers during the pre-callow stage and inhibition of contaminants seem to be two of the essential elements provided by the social environment which enable the brood to develop to adulthood. This was shown by the removal of the pupal skin by workers and the inhibition of contaminants which occurred in the controls. Progressive coloration of pupae indicating relative maturity conformed to previous studies.

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